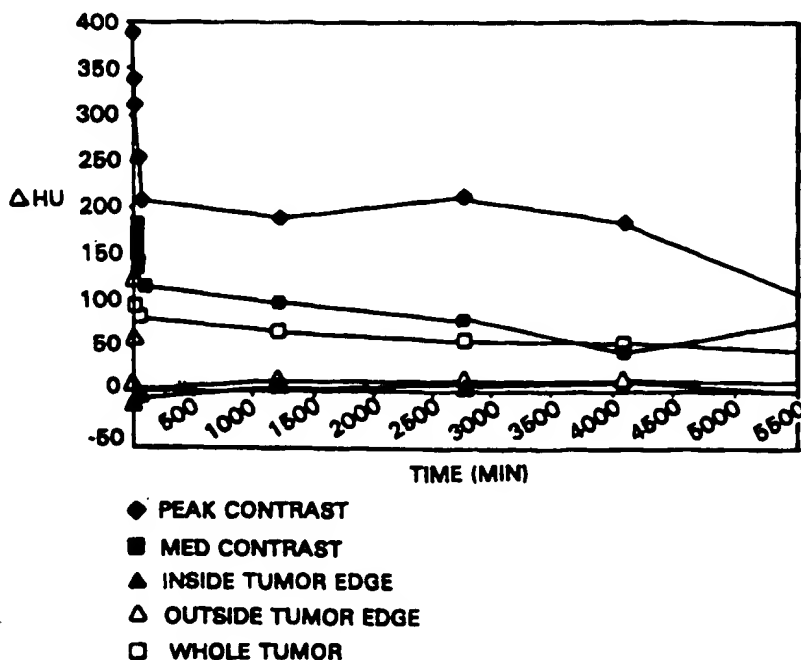


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(54) Title: **RADIODENSE COMPOSITIONS**

(57) Abstract

The invention relates to radiodense compositions for use in methods of radiation treatment of target tissues, such as tumors, the radiodense compositions include a particulate or polymeric contrast agent that has a dwell time within the target tissue of at least one hour.

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RADIODENSE COMPOSITIONSCross-Reference to Related Applications

5           This application claims priority from U.S. Patent Application Serial No. 09/183,166, filed on October 29, 1998, and U.S. Provisional Application Serial No. 60/131,418 filed on April 28, 1999, which are both incorporated herein by reference in their entirety.

10                           Field of the Invention

          The invention relates to radiodense compositions for use in methods of radiotherapeutic treatment of animate subjects, e.g., a mammalian, avian, or reptilian subjects, in particular human subjects, for example, to  
15 treat tumors.

Background of the Invention

          Radiotherapy, i.e., exposure to radiation such as  $\gamma$ -rays, X-rays and neutrons, is a well established technique used in anti-tumor therapy, especially in  
20 treating cancerous tissues that are not suitable for surgical removal. One example, X-ray phototherapy (XPT), involves the use of X-rays. In XPT a high energy X-ray beam, e.g., a 10 MV X-ray beam, is directed at the tumor. The development of CT scanners with modified collimators  
25 (CTRx - see Iwamoto et al., Radiother. Oncol., 19:337-343, 1990, and U.S. Patent No. 5,008,907 of Norman et al.) has made it possible to use CT scanners to localize tumors by CT scanning prior to and during XPT. In this technique a relatively low energy X-ray source (e.g., a  
30 140 kV source) may be used to produce a fan beam for scanning which is convertible to a pencil beam for radiotherapy.

          In such combined scanning and XPT, image contrast enhancement in CT scanning and a radiation dosage

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enhancement may be achieved by administration of an iodinated X-ray contrast agent to the subject under treatment. For example, Norman et al., Radiation Oncology Investigations, 5:8-14, 1997, describes XPT of  
5 canine brain tumors using CTRx apparatus following administration of the water-soluble low molecular weight extracellular fluid (ECF) X-ray contrast agent Renografin® (an ECF contrast agent is one which if administered parenterally distributes into the  
10 extracellular fluid).

Dose enhancement in XPT of rabbit brain tumors by administration of the water-soluble, ECF X-ray contrast agent iothalamate (Conray®) has also been reported in Iwamoto et al., Radiotherapy and Oncology, 8:161-170,  
15 1987.

Such dose enhancement is desirable since it spares normal, unaffected tissue from unnecessary radiation exposure. However, the therapeutic value of water-soluble ECF X-ray contrast agents in XPT is largely  
20 limited to the treatment of brain tumors since dose enhancement in unaffected tissue is clearly undesirable and it is essentially only within the brain that leakage of the ECF agent from the vasculature into the interstitial space of unaffected tissue is prevented. In  
25 the brain, such leakage of ECF agents into "normal" brain tissue is prevented by the blood brain barrier. In other parts of the body, ECF X-ray contrast agents can freely diffuse out of the vasculature and into all tissue compartments and may provide no discrimination between  
30 uptake of the contrast agent in tumor and "normal" tissues. Without the selective accumulation in a tumor of the X-ray contrast agent, healthy tissues would receive an undesirably enhanced radiation exposure in XPT. Since following vascular administration such ECF  
35 agents are to a large extent flushed away from the tumor

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by the blood flow, the dose enhancement is also relatively limited, e.g., corresponding to an increase of often only about 10 or 20 Hounsfield Units (HU).

Theoretical calculations have shown that every 100  
5 HU of dose enhancement in XPT roughly doubles the efficacy of the treatment in terms of its cytotoxic effect (see, T.D. Solberg, K.S. Iwamoto and A. Norman, Calculation of radiation dose enhancement factors for dose enhancement therapy of brain tumors, Phys. Med.  
10 Biol., 37(2):439-443, 1992).

#### Summary of the Invention

The present invention provides compositions and materials by which higher and more tumor-specific dose enhancement may be achieved, for example in XPT.

15 Thus, viewed from one aspect, the invention provides compositions for use in methods of enhanced radiation treatment of target tissues, such as tumors, in an animate subject (e.g., an avian, reptilian or preferably mammalian subject, in particular a human  
20 subject), wherein a cytotoxic radiation dose enhancing agent, and/or a radiation-induced cytotoxic radiation-emitting agent, is administered to the subject and a target tissue, such as a tumor, in the subject is exposed to cytotoxic radiation or cytotoxic radiation-inducing  
25 radiation, wherein the agent includes a particulate or polymeric contrast agent and is administered directly into the tumor, e.g., by injection.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as  
30 commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described

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below. All publications, patent applications, patents,  
and other references mentioned herein are incorporated by  
reference in their entirety. In case of conflict, the  
present specification, including definitions, will  
5 control. In addition, the materials, methods, and  
examples are illustrative only and not intended to be  
limiting.

The new compositions and materials provide  
numerous advantages. For example, the new compositions  
10 enable shorter therapeutic regimens and expand treatment  
options. The new compositions also enable the use of low  
energy x-rays (e.g., 20 Kev to 140 Kev) for tumor therapy  
by increasing the efficacy of the treatment itself.  
Thus, the new compositions spare normal tissue from  
15 unnecessary radiation. Furthermore, it is expected that  
more than half of all radiation treatments of cancer can  
be improved by the new compositions.

In addition, after injection of the new radiodense  
compositions, the local and any systemic distribution of  
20 the composition can be visualized in the patient using  
standard X-ray techniques, and therapy is carried out  
only if the resulting distribution is favorable.

Other features and advantages of the invention  
will be apparent from the following detailed description,  
25 and from the claims.

#### Brief Description of the Drawings

Fig. 1 is a graph comparing the change in  
radiation absorption in different parts of an  
adenocarcinoma injected with a radiodense composition  
30 (Omni-350).

Fig. 2 is a graph comparing the change in  
radiation absorption in different parts of a glioma  
injected with a radiodense composition (NI-243).

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Fig. 3 is a graph comparing the change in radiation absorption in different parts of an adenocarcinoma injected with a radiodense composition (NI-212).

5

### Detailed Description

The invention relates to new radiodense materials, agents, and compositions that provide a significant increase in the selective absorption of radiation in tumors and other diseased tissues to provide greatly enhanced methods of radiation therapy.

The radiodense compositions act as adjuvants and enhance, i.e., improve the toxic effect of, radiation therapy at locations where the composition and the radiation coexist in the proper dosage range. There is a nonintuitive relationship between the formulation and administration regimen of the radiodense composition and the external or internal radiation source as described in further detail below.

The agents and compositions for use in the invention can provide a cytotoxic radiation dose at the tumor site following exposure to radiation. In such cases, the applied radiation may itself be cytotoxic or non-cytotoxic. However, to enhance the cytotoxic efficacy of the method, preferably the applied radiation will be cytotoxic. Preferably, the agent serves to enhance the dose of applied cytotoxic radiation. In either case, the agent itself may or may not be cytotoxic, but preferably will be non-cytotoxic thereby minimizing damage to normal tissue which is not to be exposed to radiation during radiotherapy treatment. Preferably, the agent for use in the invention is a cytotoxic radiation dose enhancing agent.

The agents for use in the invention may be cytotoxic as well as X-ray contrast agents. However,

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this is not essential. The agents may, for example, become cytotoxic either through secondary radiation effects (see Norman et al., Phys. Med. Biol., 37(2):439-443, 1992), or because some population of the contrast agent is converted via incident radiation into a conventional cytotoxic molecule, or as a result of a combination of both of these effects (see Norman et al., Radiology, 129:199-203, 1978).

Preferred radiodense agents for use in the invention are those having extended residence or dwell times within the target tissue, e.g., those which may be retained within a tumor mass for up to 1 hour, or more than 2 or 5 hours, post injection. In other embodiments, the radiodense compositions can have a dwell time within the target tissue of at least 10, 15, 20, or even 24 or more hours. Certain compositions can be designed to have dwell times of several days to weeks. Particularly preferred agents include non-ECF agents. By a non-ECF agent is meant an agent which if administered into the vasculature does not leak into the interstitium. Following administration by direct injection into a tumor, a non-ECF agent cannot easily diffuse out of the tumor and is therefore substantially retained within the tumor site for a period necessary for the radiation treatment, e.g., at least 30 minutes or more post administration, preferably for one, 2, 5, 10, or more hours.

Particularly suitable agents include particulate or high molecular weight polymeric contrast agents, preferably water-soluble, high molecular weight polymers, e.g., having a molecular weight above 10 kD, preferably above 20 kD, e.g., 40 kD to 20 mD. Optionally, these polymeric agents can be used in combination with water-soluble, X-ray dense contrast agents.



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Any particulate agent which absorbs X-rays either alone or in combination with a soluble X-ray contrast agent can be used in the invention. Examples of suitable particulate agents include solid or semi-solid particles, droplets of water-immiscible liquids (e.g., oils), and vesicles (e.g., liposomes or micelles), macromolecular cage materials (e.g., materials comprising a macromolecular cage such as a carbon cage (e.g., a "buckyball" or "buckytube") with bound to or enclosed within the cage a substance which provides the cytotoxic radiation dose or dose enhancement, e.g., a heavy metal loaded buckyball or buckytube.

Suitable particulate agents can include solid particles or, alternatively, liquid or semi-solid materials embedded within, or more preferably embedded at the surface of polymeric particles. Examples of such agents include Abdoscan® (iron ferrite deposited on sulfonated styrene-divinylbenzene copolymer beads, as described in US-A-4863715 and US-A-5393525), and polystyrene beads or other polymeric particles such as polylactide co-glycolide, polylactic acid, polyurethanes, etc. with embedded metal particles. Preferred agents for use in the invention are particulate (i.e., water-insoluble) iodinated contrast agents. Such agents are well known in the art and can be readily selected. These include agents such as 3,5 bis(acetylamino)-2,4,6-triiodobenzoate (NC 8883) and 6-ethoxy-6-oxohexyl-3,5-bis(acetylamino)-2,4,6-triiodobenzoate (NC 67722) as described in Examples 5 and 10 of U.S. Patent No. 5,466,440, respectively; 1,3,5-trihexyl-2,4,6-triiodobenzene (NC 67965), as described in Example 1 of U.S. Patent No. 5,308,607, and contrast agents containing liposomes as described in WO95/26205, the disclosures of which are herein incorporated by reference in their entirety.

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Other examples of particulate, X-ray dense agents for use in the invention include barium sulfate suspensions (also an independently cytotoxic agent); Lipiodol™; Thorotrast™ (a suspension of thorium oxide);  
5 perfluorooctyl bromide (PFOB) emulsions; iron oxide particulates (e.g., known superparamagnetic particles such as AMI-227™, available from Advanced Magnetics Inc.; or those described in WO97/25073, the disclosures of which are herein incorporated by reference in their  
10 entirety), and bismuth salts. Particularly suitable for use in combination with these X-ray contrast agents are gadolinium containing agents, such as MAGNEVIST™ (gadopentetate dimeglumine), OMNISCAN™ (gadodiamide) and PROHANCE™ (gadoteridol).

15 Examples of high molecular weight molecules which may be used in the method of the invention include bulky molecules such as X-ray dense polymers, in particular dendrimers, especially starburst dendrimers. Suitable polymers include MRI active polymers (e.g., the Gd  
20 chelates NC 22181 and NC 66368 as described in WO98/23295 and WO98/10797).

Polyaminocarboxylic acid-based polymers comprising one or more iodinated aromatic moieties are particularly suitable for use in the method of the invention. Such  
25 compounds are novel and as such form a further aspect of the invention.

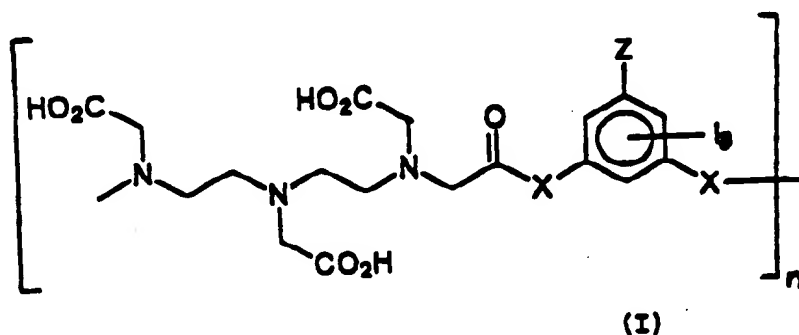
Viewed from a further aspect, the invention also provides a linear or branched polymer comprising alternating chelant and iodinated aromatic moieties, the  
30 chelant moieties including the residue of a linear polyaminocarboxylic acid or derivative thereof capable of complexing a metal ion, or a salt, or a chelate of the polymer. The chelant moieties may be the same or different and will typically be amide or ester bound to  
35 the iodinated aromatic moieties. Preferably these will

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be derived from or related to the same mono-chelant, such as EDTA, DTPA, or derivatives thereof, or other known chelating agents.

The chelating moieties present in the polymers of the invention are capable of complexing metal ions, in particular paramagnetic metal ions and heavy metal ions. Because these comprise a multiplicity of chelating sites, chelate complexes may be formed with more than one metal which may be of the same or different element.

Preferred polyaminocarboxylic acid-based polymers in accordance with the invention are those formed from one or more DTPA molecules, for example those of formula I:



wherein

X is O, NH, NR,  $(CH_2)_mNR$ , or  $CO_2(CH_2)_mNR$ , and preferably O or NH;

Z is  $CO_2H$ ,  $CO_2R$ ,  $CH_2CO_2R$ ,  $CONR_2$ , OH, OR, or a solubility enhancing group, and preferably  $CO_2H$  or OH;

R is independently hydrogen or an alkyl or aryl group, optionally substituted by one or more hydroxy, alkoxy, amine, or carboxy groups;

n is a positive integer, preferably 1-300; and m is zero or 1-8, preferably 0-4.

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The solubility of the compounds of formula I may be enhanced by varying the nature of group Z. Preferably Z is -OH, -CO<sub>2</sub>H, or a poly(alkylene oxide) moiety.

Viewed from a further aspect, the invention provides a new process for the preparation of the polymeric materials hereinbefore described, in particular those of formula I, the process including reacting one or more monochelant compounds having at least one reactive functional group with one or more iodinated aromatic moieties having at least one, preferably at least two functional groups capable of reacting with reactive groups of said monochelants, and subsequently, if required, removing any protecting groups used.

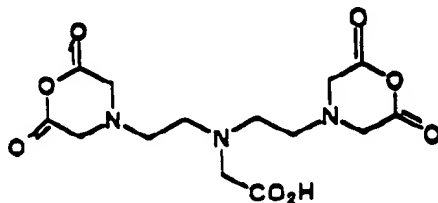
In one embodiment the process of the invention includes the following steps:

- (a) converting a polyaminocarboxylate monochelant into an activated polyaminocarboxylate compound containing one or more reactive groups, e.g., amide, anhydride, or other activated carboxyl groups;
- (b) forming an amide or ester linkage between the activated compound and an aromatic iodinated compound; and

(c) if desired, repeating steps (a) and (b) with the product of step (b) to produce a higher polymer.

In the new process, one or more of the reactive groups in the reagents can be protected during the step of forming the linkage between the compounds.

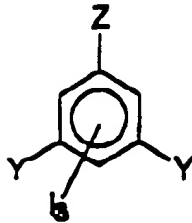
A preferred method for producing compounds of formula I includes the step of reacting a compound of formula II:



(II)

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with a compound of formula III:



(III)

wherein

Z is as hereinbefore defined; and

Y is OH, NH<sub>2</sub>, or CH<sub>2</sub>NH<sub>2</sub>.

5           Where the agent for use in the invention is particulate, the particles are conveniently of diameter 10 nm to 1000  $\mu$ m, preferably 10, 50, or 100 nm to 100  $\mu$ m. Particulate agents are conveniently formulated as suspensions, emulsions, or liposomes using standard  
10 techniques.

Any conventional surfactant may be used in forming the emulsions for use in the invention provided that the surfactant is physiologically tolerable. Such surfactants are well known to the skilled artisan and can  
15 be readily selected. The surfactants are preferably non-ionic and may be, for example, alkylene oxide polymers or copolymers, e.g., poloxamers such as the pluronics, (e.g., Pluronic F68 and F108 which are block copolymers of ethylene oxide and propylene oxide) or poloxamines  
20 such as the tetronics (e.g., tetronic 908), and the carbowaxes (which are polyethylene glycols (PEGs)), tyloxapol, polyvinylpyrrolidone, P-79 and PEG modified phospholipids. Additional useful surfactants are described in Remington's Pharmacopeia; U.S. Patents Nos.  
25 5,318,767 and 5,451,393; PCT Application WO 96/22766; and in the Handbook of Pharmaceutical Excipients.

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Nanoparticle suspensions are especially suitable for use in the invention, particularly nanoparticle suspensions of insoluble, iodinated X-ray contrast agents. These may be prepared by attrition techniques  
5 known and used in the art, including conventional roller and media milling processes. Water-insoluble X-ray contrast agents suitable for use in this aspect of the invention include water insoluble manganese particles and iodinated aromatic compounds such as iodophenoxy alkanes,  
10 iodophenyl ethers, iodophenyl esters, iodophenyl sulfonates, iodoaniline derivatives iodobenzoic acid derivatives, iodinated aryloxy carboxamides, iodinated benzoyl acetals and ketals, iodinated aryloxy esters, iodinated aromatic propanedioates and diatrizoxy esters.  
15 Such compounds are described in US-A-5318767, US-A-5401492, US-A-5346688, US-A-5476646, US-A-5424056, US-A-5484585, US-A-5360604, US-A-5531979, US-A-5472682, US-A-5560932, US-A-5662883, US-A-5716642, US-A-5260478, US-A-5330739, US-A-5322679, US-A-5466433, US-A-5472683, US-A-  
20 5384107, US-A-5264610, US-A-5525328, US-A-5521218, US-A-5500204, and WO 98/47532, WO 98/48847, WO 98/52617, and WO 97/25073, the disclosures of which are incorporated herein by reference. Particularly preferred water-insoluble X-ray contrast agents include the  
25 physiologically acceptable salts of 6-(ethoxycarbonyl)hexyl bis(3,5-acetylamino-2,4,6-tri-iodobenzoate and 1-(ethoxycarbonyl)pentyl bis(3,5-acetylamino)-2,4,6-tri-iodobenzoate.

Typically, in preparing the nanoparticle  
30 suspensions for use in the invention a surfactant wetting agent is required to achieve the desired nanoparticle size, efficacy and acceptable physical stability. Suspensions having average particle sizes of 150 nm to 300 nm may be made using a conventional surfactant or a  
35 non-ionic polymeric surfactant. Smaller average particle

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sizes may be achieved using smaller milling media, high energy milling machines, or microfluidizers.

Alternatively, nanoparticle suspensions with smaller average particle sizes, e.g., less than 100 nm, may be  
5 obtained from an insoluble compound, e.g., an insoluble iodinated X-ray contrast agent, using a wetting agent which is a soluble molecular analog of the insoluble molecule, for example in a ratio of up to 25:1, preferably up to 15:1, e.g., from 5:1 to 7.5:1. Non-  
10 ionic surfactants, e.g., Pluronic™ F108, F98, F88 or F68, and/or isotonic agents, e.g., glycerol, Isovue® or iohexol, may be used to further stabilize the resulting suspensions. In preparing suspensions of insoluble iodinated X-ray contrast agents, sodium diatrizoate,  
15 sodium metrizoate, sodium iodipamide and sodium tyropanoate are particularly suitable wetting agents.

The emulsions of the invention comprise any conventional oil phase such as an oil phase based on sesame seed oil, tall oil, sunflower oil, or coconut oil  
20 along with a surfactant as discussed above. Typically, these will be used in combination with soluble X-ray dense materials. Conveniently, the oil phase may itself be X-ray dense. For example, this may comprise a contrast agent such as Lipiodol™ (ethiodol),  
25 iophendylate, or NC 67965.

The X-ray dense agents for use in the invention can be entrapped within a liposome or associated with the encapsulating liposome shell. In such cases, the agent may be soluble or insoluble, but preferably will be a  
30 soluble contrast agent. Any conventional, physiologically tolerable, liposome may be used. Typically, the liposomes of the invention may comprise any suitable lipid, such as a phospholipid. Representative examples of useful phospholipids include  
35 lecithins (i.e., phosphatidyl-cholines), for example

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natural lecithins such as egg yolk lecithin or soya bean lecithin, and synthetic or semisynthetic lecithins such as dimyristoylphosphatidyl-choline, dipalmitoylphosphatidylcholine or distearoyl-phosphatidylcholine; phosphatidic acids; phosphatidyl-ethanolamines; phosphatidylserines; phosphatidylglycerols; phosphatidylinositols; cardiolipins; sphingomyelins; fluorinated analogs of any of the foregoing; and mixtures of any of the foregoing and mixtures with other lipids such as cholesterol.

The use of phospholipids predominantly (e.g., at least 75%) comprising molecules individually bearing net overall charge, e.g., negative charge, for example as in naturally occurring (e.g., soya bean or egg yolk derived), semi-synthetic (e.g., partially or fully hydrogenated), and synthetic phosphatidylserines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids and/or cardiolipins, may be particularly advantageous. Preferred liposomes are those having a particle size in the range of from 10 nm to 1000  $\mu\text{m}$ , preferably from 100 nm to 50  $\mu\text{m}$ .

One example of a liposomal X-ray contrast formulation suitable for use in the invention is CTP-10, a liposome of egg phosphatidylcholine and egg phosphatidylserine-sodium encapsulating iodixanol (VISIPAQUE<sup>TM</sup>) or iohexol (see WO95/26205 supra).

X-ray dense polymers suitable for use in the invention may carry one or more iodinated X-ray contrast agents such as iohexol and iodixanol. Typically, these will comprise at least 10% by weight iodine, preferably at least 20% by weight, most preferably at least 35% by weight iodine. Other suitable polymers include those carrying chelated heavy metal atoms, e.g., linear polymers carrying chelated Gd atoms as described in WO 98/23295 and WO 98/10797, or carrying any other X-ray



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absorbing moiety such as bromine, iodine or other heavy atoms such as tungsten, calcium, barium, bismuth etc. Dendrimeric moieties carrying X-ray absorbing atoms are especially preferred. Polymers carrying heavy metal atoms will generally comprise at least 10%, preferably at least 20%, e.g., at least 35% by weight heavy metal.

Conventional MRI contrast agents which incorporate paramagnetic metal species having medium to high atomic weights are X-ray dense. Linked to a high molecular weight molecule, e.g., a linear, branched or dendrimeric polymer, especially preferably a dendrimer, for example a starburst dendrimer, such contrast agents are suitable for use in the invention. Examples of suitable MRI contrast agents include chelates of paramagnetic species, e.g., Gd(III), Mn(II), Cr(III), Dy(III) and Fe(III) with cyclic or acyclic polyaminocarboxylic acids such as DTPA, DTPA-bismethylamide, DOTA, DO3A, HP-DO3A and derivatives thereof. Other suitable paramagnetic metal chelates and polymers carrying such species are disclosed for example in EP-A-71564, EP-A-130934, DE-A-3401052, EP-A-185899, EP-A-186947, EP-A-165728, US-A-4647447, US-A-4826673, EP-A-299795, EP-A-258616, DE-A-3633245, EP-A-263059 and EP-A-277088 EP-A-232751, EP-A-230893, EP-A-292689, EP-A-287465, DE-A-3633245, DE-A-3324235, EP-A-250358, EP-A-263059, EP-A-173163, EP-A-255471, US-A-4639365, US-A-4687659, WO86/02005, WO87/02893, WO85/05554, WO87/01594, WO87/06229, WO90/08134 and WO90/08138 and in the documents cited in these patent publications, the disclosures of which are incorporated by reference.

The agents and compositions of the invention additionally may be or contain a cytotoxic agent to enhance the cytotoxic efficacy of anti-tumor methods. Such a cytotoxic agent may be conjugated to, or contained within, a particulate agent, or conjugated to a water-soluble agent, or can be free in the solution phase

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around the particulate. Where a cytotoxic agent is conjugated to the agent, this is preferably via a biodegradable covalent bond, e.g., via an amide, ester, or double ester function. When using an emulsion of an X-ray dense material, an insoluble cytotoxic agent is preferably present, i.e., dissolved in, the oil phase of the emulsion.

Suitable cytotoxic agents include conventional oncology therapeutic drugs, particularly those employed in brachytherapy, such as radioactive moieties, e.g., indium oxime,  $^{103}\text{Pd}$  or  $^{125}\text{I}$  containing seeds,  $^{90}\text{Y}$ ,  $^{32}\text{P}$  and other conventional radioactive nuclei.

The agent for use in the invention may further comprise an amount, preferably a significant amount (greater than 50%), of a soluble X-ray dense material, e.g., a water-soluble X-ray contrast agent. Such materials can be iodinated, ionic or non-ionic materials, for example non-ionic monomers, ionic monomers, non-ionic dimers and ionic dimers. Such monomers or dimers will generally contain iodophenyl moieties, e.g., diiodophenyl or more preferably triiodophenyl moieties and may contain one or more, e.g., 1, 2, or 3 such iodophenyl groups per molecule. The iodophenyl rings will in such an event generally be substituted by solubilizing groups, e.g., ionic groups such as groups containing mono or polyhydroxy- $\text{C}_{1-10}$ -alkyl moieties optionally linked to the phenyl ring via an amide function. A wide variety of such iodophenyl agents are known from the literature and examples include the X-ray contrast agents iohexol, iopamidol, ioversol, iopentol, iodixanol, iopromide, iomeprol, iosimide, metrizamide, iotasol, iotrolan, iodecimol, ioglucol, iogluamide, iosarcol, iotasul, ioxilan, iodamide, iothalamate, diatrizoate, ioxaglate and metrizoate. Of particular importance are water-soluble, X-ray contrast agents of low molecular weight

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such as iohexol (Omnipaque®), iopamidol (Isovue®), diatrizoate (Hypaque®), iopentol, ioxilan, and iodixanol.

An especially preferred formulation for use in the invention is one prepared from small nanoparticles of an insoluble contrast agent, e.g., an iodinated X-ray contrast agent, using a soluble, close structural relative of the same molecule as a wetting agent. Such suspensions typically demonstrate average particle sizes less than 100 nm with 100% of the particles having a diameter less than 200 nm. For example, such formulations may be prepared by wet milling of the contrast agent with the corresponding soluble molecular analog to produce very small particles (average size < 100 nm) which, following the addition of a surfactant, e.g., a non-ionic polymeric surfactant, or a significant amount of a soluble X-ray contrast agent, can be sterile filtered.

The agents and compositions of the invention can be radiation dose enhancing compositions used in conjunction with exposure of the subject to cytotoxic radiation, e.g., X-rays, and/or the agents and compositions can include a material which, when exposed to radiation (e.g., neutron radiation) which may or may not be cytotoxic, itself emits cytotoxic radiation. Thus, the compositions may for example be X-ray opacifiers or neutron capture therapy agents, such as boron. Preferably the agent is a material which effectively absorbs X-rays and emits energetic radiation, e.g., electrons,  $\alpha$ -particles, photons (e.g.,  $\gamma$ -rays), and/or heat sufficient to damage neighboring cells, i.e., cells within the region of the agent. Thus, the agents for use in the invention do not function via chemical toxicity, but rather as a result of a secondary shower of energetic particles and/or heat which is released upon absorption of the incident radiation, e.g., X-ray beam.

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These energetic particles and/or heat are directly responsible for the cellular damage, which ultimately leads to death of any tumor cells, while "normal" (i.e., healthy) cells are able to recover from the particle  
5 and/or heat mediated damage.

Radiation for use in the method of the invention may be cytotoxic or non-cytotoxic. Preferably, this will be cytotoxic ionizing radiation, typically X-rays or  $\gamma$ -rays. Megavoltage radiation (e.g., derived from X-ray  
10 generators having tube potentials in excess of 250 kV, e.g., up to 3000 kV) can be useful in treating deep tumors. Low energy X-rays in the orthovoltage region (e.g., derived from X-ray generators having tube potentials < 250 kV, preferably in the range 80 to 150  
15 kV, for example 120 to 140 kV) are also useful.

Orthovoltage X-rays have the advantage that these cause much less damage to surrounding healthy tissue.

During radiotherapy the tumor mass will, in general, be irradiated from multiple directions thereby  
20 concentrating the radiation dose on the tumor. To be effective, accurate mapping (e.g., by CT scanning) of tumor size and location is critical prior to radiotherapy (see, e.g., U.S. Patent No. 5,008,907 of Norman et al.).

CT scanners modified with the addition of a  
25 collimator (CTx) which converts a fan beam to a pencil beam are capable of delivering radiation therapy with great precision and can be used in the invention. In this regard, the CT scanner has two functions: to help localize the tumor prior to radiotherapy and to increase  
30 the absorbed radiation dose during radiotherapy. The patient can therefore be scanned and treated using the same equipment.

The compositions of the invention find particular use in radiotherapy of tumors and other tissues,  
35 especially in the radiation treatment of tumors and

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metastases. Preferably, the invention can be used to enhance radiation therapy in the treatment of any solid tumor, especially in treating tumors of the prostate, breast, lung, head, neck and brain, in treating liver  
5 lesions, and in the prevention of metastases (e.g., lymph node metastases). In this regard, the radiosensitizers described herein provide a number of important advantages over the use of conventional contrast agents, e.g., in terms of degree of enhancement of applied radiation  
10 (i.e., efficacy) and increased residence time within the tumor. Increased enhancement of radiation energy not only maximizes the impact of the treatment on the tumor, but also reduces the required dosage of the radiosensitizer. Levels of radiation enhancement  
15 achievable using the agents of the invention are typically in excess of 20, 50, or 100 HU, preferably > 200 HU, more preferably > 1000 HU and most preferably > 2000 HU. Increased residence time of the contrast agent within the tumor also avoids the need for repeated dosage  
20 of the agent when carrying out further radiation treatment.

The X-ray dense materials described herein can be used to advantage in any conventional method of brachytherapy with increased efficacy. Specifically, the  
25 choice of X-ray dense material may be tailored to the particular type of brachytherapy, including neutron capture therapy (e.g., when using boron compounds as the contrast agent), in treating prostate tumors (e.g., using RAPID STRAND™, available from Nycomed Amersham, Little  
30 Chalfont, UK, in conjunction with external beam X-ray radiation), and in treating certain brain tumors (e.g., in which radiation from a pencil probe is delivered directly into the tumor).

Viewed from a further aspect, the invention  
35 provides the new radiation-induced cytotoxic radiation-

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emitting agents and/or cytotoxic radiation dose enhancing agents as hereinbefore described, for the manufacture of a therapeutic agent for use in enhanced radiation therapy methods.

5           The dosage of the agent used in the method of the invention will vary according to the precise nature of the agent used, the intended administration route and the machine parameters such as intended X-ray electron voltage. Preferably however the dosage should be kept as  
10 low as is consistent with achieving a satisfactory radiation dose. Dosages of from 1 to 100 mmol/kg bodyweight, preferably of from 3 to 500 mmol/kg bodyweight, e.g., of from 5 to 50 mmol/kg bodyweight are particularly preferred. It is well within the skill of  
15 the average practitioner in this field to determine the optimum dosage for any particular agent by relatively routine experimentation, either in vivo or in vitro.

          The agents may be formulated with conventional pharmaceutical or veterinary aids, for example  
20 stabilizers, osmolality adjusting agents, buffers, pH adjusting agents, etc. and may be in a form suitable for injection directly or after dispersion in or dilution with a physiologically acceptable carrier medium, e.g., water. Thus the agents for use in the invention may be  
25 formulated in conventional administration forms such as solutions, suspensions, dispersions etc. in a physiologically acceptable carrier medium.

          Parenterally administrable forms, e.g intravenous solutions, should of course be sterile and free from  
30 physiologically unacceptable agents, and should preferably have low osmolality to minimize irritation or other adverse effects upon administration. Thus the agent for administration should preferably be isotonic or slightly hypertonic. Both hypertonic and hypotonic  
35 solutions may, however, be used. Suitable vehicles for

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administration include aqueous vehicles conventionally used for administering parenteral solutions such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, 5 Lactated Ringer's Injection etc. The solutions may contain preservatives and antimicrobial agents conventionally used for parenteral solutions, excipients and other additives which are compatible with the agent and which will not interfere with the manufacture, 10 storage or use of products.

Preferably the agents for use in the invention will be in a form suitable for direct injection into the tumor mass. Alternatively, these may be in a form suitable for surgical implantation (e.g., as RAPID 15 STRAND™ iodine seeds).

#### EXAMPLES

The invention will now be further described with reference to the following non-limiting example.

##### Example 1

20 A nanoparticle suspension of NC 67722 (6-(ethoxycarbonyl)hexyl bis(3,5-acetylamino-2,4,6-tri-iodobenzoate) was prepared with wet milling using the corresponding carboxylic acid, NC 68056 (sodium 6-[(3,5-bis(acetylamino)-2,4,6-tri-iodophenyl)carbonyloxy] 25 hexanoate), and NaOH as auxiliary wetting agents.

In a 100 mL Pyrex bottle containing 50 mL of zirconium silicate ( $\text{ZrSiO}_4$ ) beads (0.3, 0.5, 0.7 or 1.1 mm) or 0.5 mm Yttrium-doped zirconia milling beads, suspensions consisting of 25% (w/v) NC 67722, 5% (w/v) NC 30 68056, 5% non-ionic surfactant and NanoPure water were prepared. NC 68056 was added as the free acid (such that the sodium salt was formed in situ from NaOH and the free acid during milling) or as the sodium salt. The final pH

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was adjusted by the addition of 1N HCl. The mixture was then roller milled at optimal speed until the desired particle size was obtained (< 7 days).

Once milled, the suspension was recovered from the  
5 beads and filtered through sequentially smaller filters until final filtration through 0.2  $\mu$ m could be attempted. The resulting suspension was then diluted with a presterilized stock solution of Pluronic F98 (BASF) and glycerol.

10 The final formulation comprised 15% (w/v) NC 67722, 3% (w/v) NC 68056, 3% (w/v) Pluronic F98, 1.75% (w/v) glycerol and contained 180 mg iodine per ml. The average particle size (as determined by light scattering) was 97 nm. 100% of particles were smaller than 197 nm.  
15 The pH of the formulation was determined to be 8.2, with an osmolality of 266 mOsm/kg as determined by vapor pressure osmometry. This formulation is designated NI-244 (Nycomed), and is suitable for lymph node and intratumoral applications.

20 Chemical and physical stabilities of the nanoparticle suspension were estimated on the basis of average particle size, particle size distribution, pH, osmolality and concentration of NC 67722 and NC 68056 as measured during a period of 8 months at both ambient  
25 temperature and 5°C. No change was noted in any parameter during these 8 months.

#### Example 2 - Combinations of Low Molecular Weight and High Molecular Weight Radiodense Materials

Various radiodense compositions have been prepared  
30 that include both low molecular weight radiodense materials and high molecular weight radiodense materials. For example, NI-243 combines Win 8883 with water soluble iohexol; NI-212 combines NC 72144 (ethyl 3,5-dihexyl-2,4,6-triiodophenoxyacetate) with iohexol; and NI-244



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combines NC 67722 with NC 68506 in ratios of approximately 5 parts high molecular weight material to 1 part low molecular weight material (wt/vol). These ratios are selected depending upon their intended purpose. In particular, it is easy to add more water-soluble, low molecular weight material such as iohexol if it is intended to create a high, relatively short-lived, enhancement of radiation absorption in a tumor through intratumoral injection.

10 Other radiodense compositions that have similar characteristics include liposomal compositions containing equal amounts of water-soluble, low molecular weight radiodense materials inside and outside the liposome. The size of the liposome can be varied as well as the composition of the lipid membrane. The encapsulated material can be one of several known water-soluble, low molecular weight radiodense materials such as iohexol, iopamidol, iomeprol, iodixanol, or ioversol, or combinations thereof.

20 Other radiodense compositions are micellar block co-polymers such as those described in U.S. Patent No. 5,567,410. As described above, these micellar compositions can be enriched by the addition of water soluble, low molecular weight radiodense materials.

25 Example 3 - Dwell Times of Radiodense Compositions in Vivo

Immunologically tolerant mice were implanted with several different human neoplasms. When the tumors reached a size of 1-2 cm, radiodense compositions were injected intratumorally via a percutaneous route using a 27 gauge needle. The mice and their tumors were then serially imaged with computed tomography to determine the local pharmacokinetics of the injectates by measuring the x-ray attenuation (in HU) of regions of interest.

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Fig. 1 is a plot of the temporal change of x-ray attenuation in a mouse bearing a human adenocarcinoma (LS174T). The radiodense material was a water-soluble, low molecular weight radiodense material (Omnipaque™ with a concentration of 350 mg I/ml as iohexol). A peak contrast of nearly 3000 HU was attained in the center of the tumor, with smaller degrees of contrast enhancement surrounding the injected area. This low molecular weight material was rapidly cleared with values of only 2000 HU at the peak location 60 minutes later.

Fig. 2 shows a similar experiment in which the human tumor was a glioma (U87-VC2) and the intratumor injectate was NI-243. In this example, the low molecular weight material created a peak contrast of about 1300 HU, with a rapid washout over 60 minutes. The high molecular weight material sustained a concentration of about 500 HU for more than 1 day.

Fig. 3 shows a third experiment of this kind where the mouse was implanted with an adenocarcinoma (LS174T) and the intratumor injectate was NI-212. A smaller volume was administered and the temporal graph shows a high peak contrast that disappears with about the same clearance rate as the iohexol above, but the high molecular weight material concentration (in the peak area) was sustained at 200 HU for about 3 days.

Example 4 - In Vivo Study of Diffusion of Low Molecular Weight and High Molecular Weight Radiodense Materials After Intratumoral Injection

About 200 µl of either a low molecular weight radiodense material (iohexol) or a high molecular weight radiodense material (WIN 8883) was injected directly into separate VX2 tumors growing in the thigh of a rabbit. To measure the diffusion time of the two materials in the tumor interstitium, the rabbit was euthanized and the

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corpus imaged using CT over the next 24 hours to measure the spatial and temporal distribution of each material.

As shown in the Table below, there was very little expansion of the volume of the high molecular weight radiodense material over 21 hours. On the other hand, the volume of the low molecular weight radiodense material expanded rapidly beyond the initial injection locus over the same 21 hour time period. The Table below shows the volume of each material over time in units of total number of intratumoral voxels containing at least 200 HU.

Table

Win 8883 Volume

Omni 350 Volume

Initial	10420	12722
1.5 Hrs	10898	23912
4.5 Hrs	11826	22104
21.0 Hrs	11682	44720

These results show that the high molecular weight radiodense material is indeed trapped within the tumor, and remains active to enhance radiation absorption, for an extended period of time of at least 21 hours.

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Claims

1. A radiodense composition for use in a method of radiation treatment of a target tissue in an animate subject, the radiodense composition comprising a  
5 particulate or polymeric contrast agent that has a dwell time within the target tissue of at least one hour, and wherein the radiodense composition is administered directly into the target tissue, and the target tissue is thereafter exposed to radiation.
- 10 2. The composition of claim 1, wherein the composition comprises a radiation-induced cytotoxic radiation-emitting agent.
3. The composition of claim 1, wherein the composition comprises a cytotoxic radiation dose  
15 enhancing agent.
4. The composition of claim 1, wherein the radiodense composition comprises iohexol, iopamidol, ioversol, ioxilan, iomeprol, or iodixanol.
5. The composition of claim 1, wherein the  
20 radiodense composition comprises iodine, barium, bismuth, boron, bromine, calcium, gold, silver, iron, manganese, nickel, gadolinium, dysprosium, tungsten, tantalum, stainless steel, or nitinol, or a combination of any one or more of the above.
- 25 6. The composition of claim 1, wherein the radiodense composition comprises a radiodense material present within a small, lipid soluble molecule.

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7. The composition of claim 1, wherein the radiodense composition has a dwell time within the target tissue of at least 5 hours.

8. The composition of claim 1, wherein the  
5 radiodense composition has a dwell time within the target tissue of at least 24 hours.

9. The composition of claim 1, wherein the radiodense composition is about 10 nanometers to 100 microns in size.

10 10. The composition of claim 1, wherein the radiodense composition comprises NI-244, NI-212, or a liposome comprising iohexol.

11. The composition of claim 1, wherein the composition further comprises a soluble X-ray dense  
15 material.

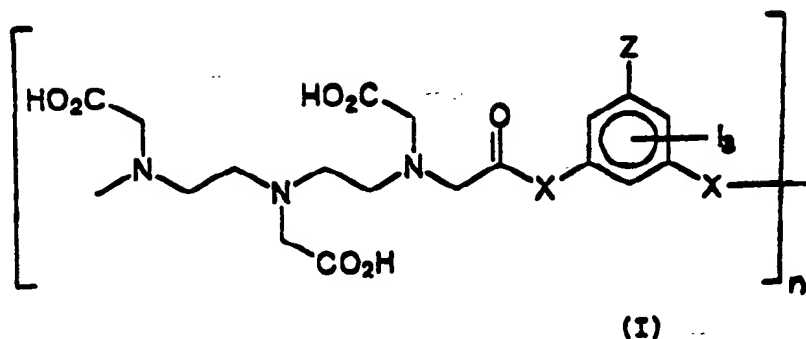
12. The composition of claim 1, wherein the target tissue is a tumor.

13. The use of a radiodense composition of claim 1 in the manufacture of a therapeutic agent for use in a  
20 method of radiation therapy of a target tissue.

14. A linear or branched polymer comprising alternating chelant and iodinated aromatic moieties, the chelant moieties comprising the residue of a linear polyaminocarboxylic acid or derivative thereof capable of  
25 complexing a metal ion, or a salt or chelate of the polymer.

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15. A polymeric composition comprising formula I:



wherein

X is O, NH, NR,  $(CH_2)_mNR$ , or  $CO_2(CH_2)_mNR$ ;

Z is  $CO_2H$ ,  $CO_2R$ ,  $CH_2CO_2R$ ,  $CONR_2$ , OH, OR, or a  
5 solubility enhancing group;

R is independently hydrogen or an alkyl or aryl group, optionally substituted by one or more hydroxy, alkoxy, amine, or carboxy groups;

n is a positive integer; and

10 m is zero or 1 to 8.

16. The composition of claim 15, wherein the composition is a polyaminocarboxylic acid-based polymer.

17. The composition of claim 15, wherein the solubility of the compound is enhanced by varying the  
15 nature of group Z.

18. The composition of claim 15, wherein X is O or NH.

19. The composition of claim 15, wherein Z is  $CO_2H$  or OH.

20. The composition of claim 15, wherein Z is a poly (alkylene oxide) moiety.

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21. The composition of claim 15, wherein n is 1 to 300.

22. The composition of claim 15, wherein m is 0 to 4.

5           23. A method for preparing a polymeric composition, the method comprising  
            reacting one or more monochelant compounds having  
            at least one reactive functional group with one or more  
            iodinated aromatic moieties having at least one  
10 functional group capable of reacting with reactive groups  
            of said monochelants.

24. The method of claim 23, wherein the iodinated aromatic moieties each comprise at least two functional groups.

15           25. The method of claim 23, wherein the method is used to prepare the polymeric composition of claim 7.

26. A method of preparing a polymeric composition, the method comprising  
            (a) converting a polyaminocarboxylate monochelant  
20 into an activated polyaminocarboxylate compound  
            containing one or more reactive groups; and  
            (b) forming an amide or ester linkage between the  
            activated polyaminocarboxylate compound and an aromatic  
            iodinated compound to form the polymeric composition.

25           27. The method of claim 26, further comprising  
            (c) repeating steps (a) and (b) with the product  
            of step (b) to produce a higher polymer.

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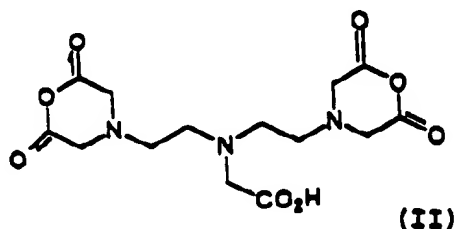
28. The method of claim 26, further comprising protecting one or more of the reactive groups in the activated polyaminocarboxylate compound during the step of forming the linkage between the compounds.

5 29. A method of claim 26, wherein the reactive groups in the activated polyaminocarboxylate compound comprise an amide or anhydride.

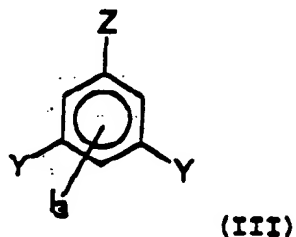
30. A method of claim 26, wherein the reactive groups in the activated polyaminocarboxylate compound  
10 comprise an activated carboxyl group.

31. The method of claim 26, wherein the method is used to prepare the polymeric composition of claim 8.

32. A method for preparing a polymeric composition, the method comprising  
15 reacting a compound of formula II:



with a compound of formula III:



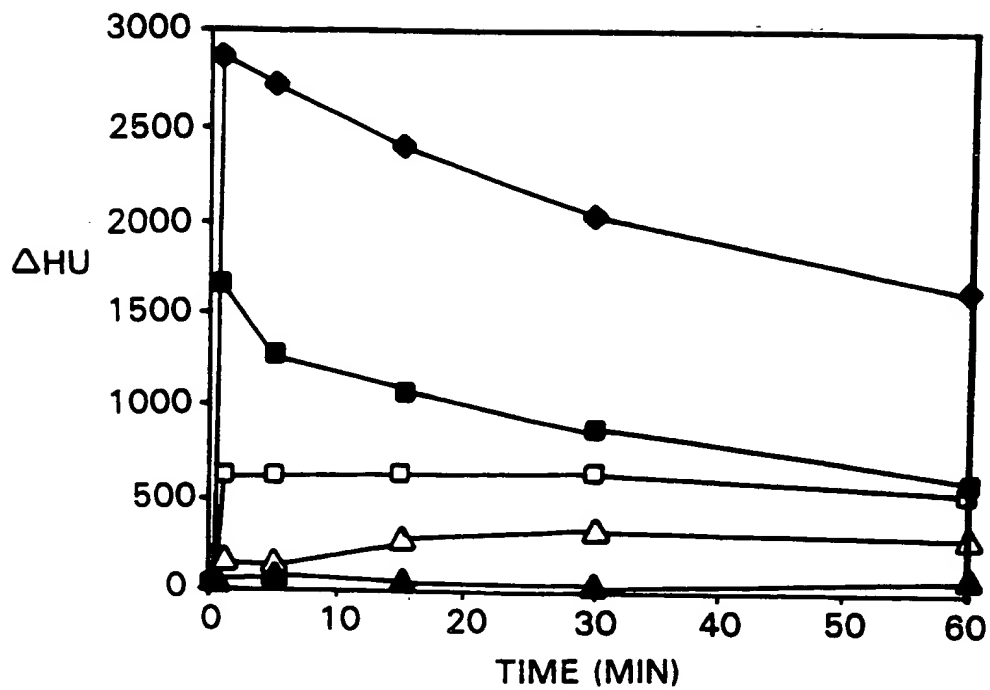
wherein

Z is CO<sub>2</sub>H, CO<sub>2</sub>R, CH<sub>2</sub>CO<sub>2</sub>R, CONR<sub>2</sub>, OH, OR, or a solubility enhancing group; and

20 Y is OH, NH<sub>2</sub>, or CH<sub>2</sub>NH<sub>2</sub>).



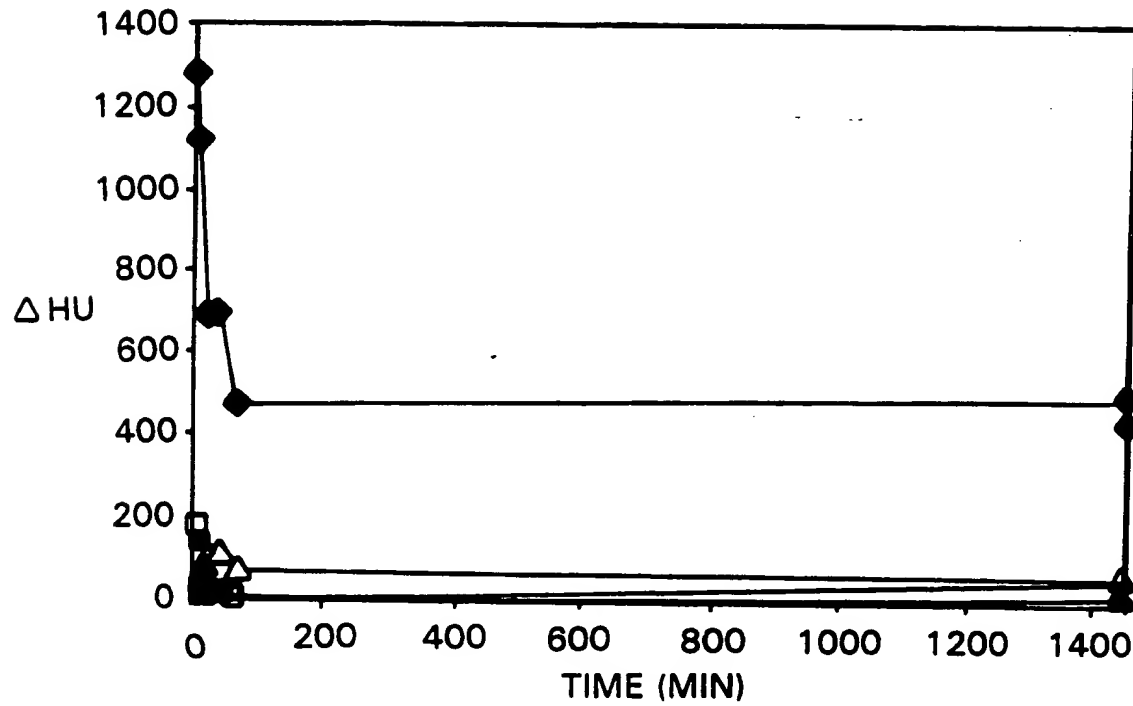
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- ◆ PEAK CONTRAST
- MED CONTRAST
- ▲ INSIDE TUMOR EDGE
- △ OUTSIDE TUMOR EDGE
- WHOLE TUMOR

**FIG. 1**

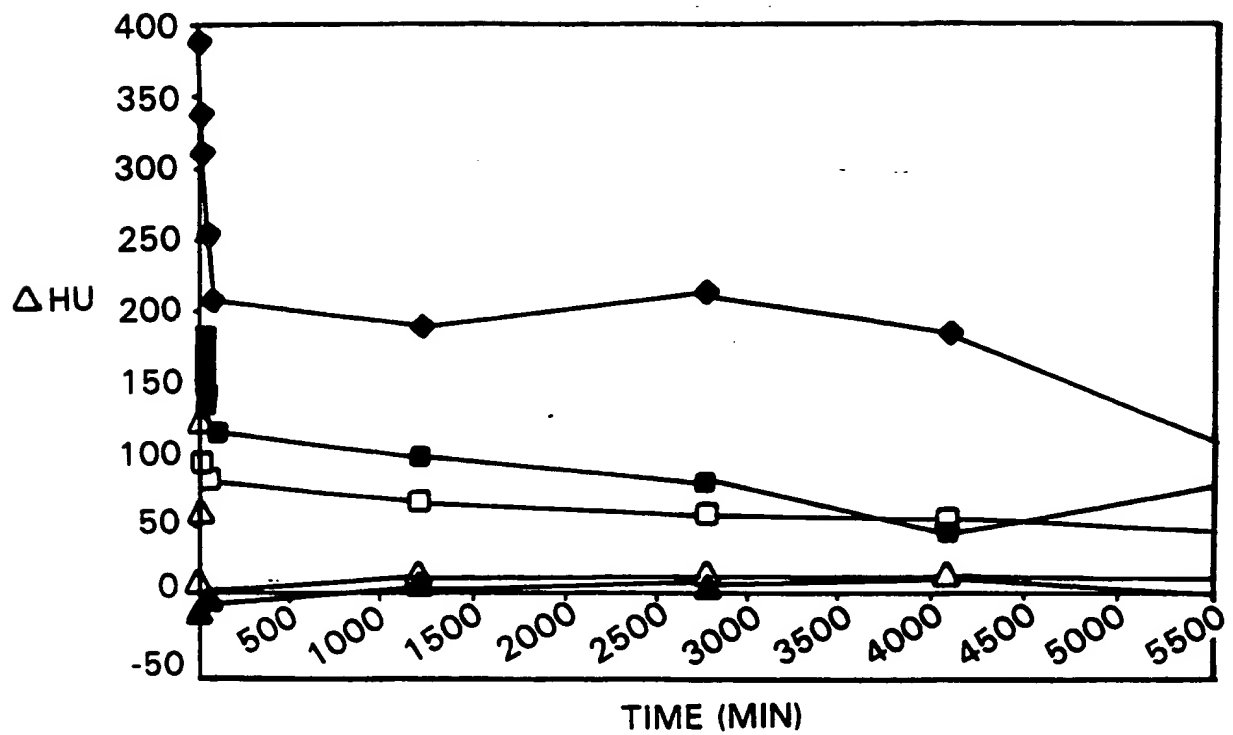
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- ◆ PEAK CONTRAST
- MED CONTRAST
- ▲ INSIDE TUMOR EDGE
- △ OUTSIDE TUMOR EDGE
- WHOLE TUMOR

**FIG. 2**

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- ◆ PEAK CONTRAST
- MED CONTRAST
- ▲ INSIDE TUMOR EDGE
- △ OUTSIDE TUMOR EDGE
- WHOLE TUMOR

FIG. 3